

Claims

1. A method for maturation of conifer somatic embryos, comprising
- 5 a step, where an embryogenic cell mass is cultured with a culture medium comprising an anti-auxin.
- 10 2. A method according to claim 1, further comprising a second step before the anti-auxin step, where the embryogenic cell mass is cultured with a culture medium.
- 15 3. A method according to claim 1, further comprising a third step after the anti-auxin step where the embryogenic cell mass is cultured with a culture medium essentially free of anti-auxin.
- 20 4. A method according to claim 1, whereby the anti-auxin step lasts from 2 days to 50 weeks.
- 25 5. A method according to claim 2, whereby the second step before the anti-auxin step lasts from two days to 10 weeks.
- 30 6. A method according to claim 3, whereby the third step after the anti-auxin step lasts from two days to 40 weeks.
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~~7. A method according to any of the preceding claims, whereby the culture medium in at least one of the steps further comprises a maturation agent.~~
- ~~8. A method according to claim 7, whereby the culture medium of all the steps further comprises at least one maturation agent.~~
- ~~9. A method according to claim 7, whereby the maturation agent is selected from the group comprising abscisic acid, silver nitrate, jasmonic acid, abscisyl alcohol, acetylenic aldehyde, dihydroacetylenic alcohol, phaseic acid, dihydrophaseic acid, 6'-hydroxymethyl abscisic acid, beta-hydroxy abscisic acid, beta-methylglutaryl abscisic acid, beta-hydroxy-beta-methylglutarylhydroxy abscisic acid, 4'-desoxy ab-~~

scisic acid, abscisic acid beta-D-glucose ester, 2-2(2-p-chlorophenyl-trans-ethyl)cyclopropane carboxylic acid.

Side A 2
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10. A method according to any of the claims 7 to 9, whereby the maturation agent is abscisic acid.

11. A method according to claim 10, whereby the concentration of abscisic acid is between 0.1 and 200 μ M.

10 12. A method according to claim 1, whereby the anti-auxin is selected from the group α -(1-naphthylmethyl-sulfide)-isobutyric acid, α -(1-naphthylmethyl-sulfide)-propionic acid, α -(2-naphthylmethyl-sulfide)-isobutyric acid, α -(2-naphthylmethyl-sulfide)-propionic acid, δ -(naphthylmethyl-selenide)- η -valeric acid, (-)- α -(2,4,5-trichlorophenoxy)-propionic acid, (-)- α -(2,4-dichlorophenoxy)-propionic acid, (-)- α -(2-naphthoxy)-propionic acid, (+)- α -(1-naphthoxy)-propionic acid, (3-phenyl, 1,2,4-thiadiazol-5-yl)thioacetic acid (PTAA), β -naphthalene acetic acid (β -NAA), γ -phenylbutyric acid, 1-(naphthylmethyl-sulfide)-propionic acid, 1-naphthylmethyl-selenidacetic acid, 2-(naphthylmethyl-sulfide)-propionic acid, 2-(o-chlorophenoxy)-2-methylpropionic acid, 2,3,4,5,6-pentachlorophenoxyisobutyric acid, 2,3,5-triiodobenzoic acid (TIBA), 2,3,5-triiodobenzoic acid, 2,4,5-trichlorophenoxyisobutyric acid, 2,4,6-trichlorophenoxyacetic acid (2,4,6-T), 2,4,6-trichlorophenoxyisobutyric acid, 2,4-dichloroanisole (2,4-DCA), 2,4-dichlorophenoxyisobutyric acid (2,4-DCIP), 2,4-dichlorophenylsulfoneacetic acid, 2,4-dichlorophenylsulfoxideacetic acid, 2,6-dichlorophenoxyactic acid, 2-chlorophenoxyisobutyric acid, 2-naphthylmethyl-selenidacetic acid, 3-chlorophenoxyisobutyric acid, 3-indoleisobutyric acid, 3-nitro-4-fluorobenzoid acid, 4-chlorophenoxyisobutyric acid, 5-methyltryptophan, 7-aza-indol, 9-hydroxyfluorene-9-carboxylic acid (HFCA), ferulic acid, flavonoids, indole-isobutyric acid, kaempferol, maleic hydrazide, naptalam (N-1-naphthylphthalamic acid), p-Chlorophenoxyisobutyric acid (PCIB), p-coumaric acid, phenoxyacetic acid, phenoxyisobutyric acid, phenylpropionic acid, quercitin, trans-cinnamic acid.

13. A method according to claim 1, whereby the anti-auxin is PCIB.

35 14. A method according to claim 1, whereby the anti-auxin is PCIB at a concentration between 0.01 and 200 μ M.

15. A method according to claim 1, whereby the anti-auxin is PCIB at a concentration between 1 and 50 μ M.

5 16. A method according to claim 1, whereby the conifer is a member of the Pinaceae.

10 17. A method according to claim 1, whereby the conifer is selected from the genera *Pinus*, *Picea*, *Abies*, *Larix*, and *Pseudotsuga*.

Sub A3 18. A method according to claim 1, whereby the conifer is an *Abies* sp such as *Abies nordmanniana*.

15 19. A method according to claim 1, whereby the conifer is a *Picea* sp such as *Picea abies* or *Picea sitchensis*.

20 20. A method according to claim 1, whereby the conifer is an *Abies* sp such as *Abies nordmanniana* and the anti-auxin is PCIB at a concentration between 1 and 100 μ M.

21. A method according to claim 1, whereby the conifer is a *Picea* sp and the anti-auxin is PCIB at a concentration between 0.1 and 50 μ M.

25 22. A method according to claim 3, whereby the culture medium used during at least part of the third step after the anti-auxin step further comprises an auxin.

23. A method according to claim 22, whereby the concentration of the auxin in the culture medium is between 0.001 and 100 μ M.

30 24. A method according to claim 22 or 23, whereby the auxin is selected from the group comprising indole acetic acid, indolebutyric acid, napthalene acetic acid, 2,4-D, 2-naphthoxyacetic acid (NOA), 4-chlorophenoxyacetic acid (4-CPA), 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 3,6-dichloroanisic acid (dicamba), 4-amino-3,5,5-trichloropicolinic acid (picloram), otonil, 2-chloro-3(2,3-dichloro-phenyl)-propionitril (CDPPN),

25. A method according to any of the claims 22 to 24, whereby the at least part of the third step comprising an auxin lasts from two days to 40 weeks.

5 26. A method according to claim 3, whereby the embryogenic cell mass is being further cultured with a culture medium comprising metabolisable carbon sources.

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27. A method according to claim 3, whereby the embryogenic cell mass is being further cultured with a culture medium comprising carbohydrate sources.

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28. A method according to claim 27, whereby the embryogenic cell mass is being further cultured with a culture medium comprising sucrose.

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29. A method according to claim 27, whereby the embryogenic cell mass is being further cultured with a culture medium comprising fructose.

30. A method according to claim 27, whereby the embryogenic cell mass is being further cultured with a culture medium comprising glucose.

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31. A method according to claim 27, whereby the culture medium has a content of between 1 and 100 g/L of metabolisable carbon sources.

32. A method according to claim 27, whereby the further culturing is performed for a period of from 2 days to 10 weeks.

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33. A mature conifer somatic embryo produced by the method according to any of the preceding claims.

34. An embryo according to claim 33 having a water content less than 70 %.

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35. An embryo according to claim 33 being transgenic and comprising recombinant DNA sequences.

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36. A conifer plant produced from an embryo according to any of the claims 33 to 35.

37. A plant according to claim 36 being transgenic and comprising recombinant DNA sequences.

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